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MINIREVIEW

Colonization Resistance

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In 1987 we reviewed the literature on the concept of colonization resistance (CR) (12). In this concept, the indigenous anaerobic flora limits the concentration of potentially pathogenic (mostly aerobic) flora in the digestive tract. This implies that the risk of superinfections by aerobic flora would be eliminated by selecting those antimicrobial agents which spare the anaerobic flora. The concept of CR was based on experiments in animals and uncontrolled observations in patients. It was not validated conclusively.

Since publication of our previous review (12), we have improved the design for studying the influence of antimicrobial agents on CR, especially by using human volunteers and analyzing the data in each volunteer separately with single-patient statistics (57, 63). This methodology was applied in the study of several antimicrobial agents (57–61, 63, 64). The resulting data provide a better understanding of the impact of antimicrobial agents on the microbial flora of the bowel. At present, it seems to be the case that some former conclusions were made prematurely, especially those pertaining to the availability of antimicrobial agents that do not impair CR (12, 47).

This review will be restricted to a discussion of CR of the bowel.

THE NORMAL FLORA OF THE BOWEL

The normal flora of the bowel contains more than 400 obligate anaerobic species (35) in a total concentration of 10^{11} to 10^{12} CFU/g of feces. The concentration of this aerobic flora is much lower, and the number of these species in the bowel is small. In our healthy volunteers, the median concentration of aerobic gram-negative bacilli (GNB) and aerobic gram-positive cocci in feces was $\leq 10^8$ CFU/g and the median concentration of yeasts in feces was $\leq 10^3$ CFU/g (57–61, 63, 64). Among the GNB in the bowel, *Escherichia coli* is dominant.

Large numbers of GNB other than *E. coli* (secondary GNB) are ingested daily with food, especially salads (13, 40, 68). Yet these secondary GNB fail to colonize the bowel in most people. It appears that exogenous strains of *E. coli* are more successful than secondary GNB in colonizing the bowel in healthy volunteers (2, 36, 42). Some strains of *E. coli* may persist for months or years, while others have a limited tenure of a few days or weeks (2, 42).

Among the aerobic gram-positive cocci, the enterococci are dominant. Apart from enterococci, staphylococci or streptococci colonized the bowel in some of our volunteers at low concentrations ($\leq 10^5$ CFU/g of feces) (57–61, 64).

Although less than 0.1% of the normal flora consists of aerobes, most endogenous infections are caused by aerobic flora. According to Dubos (16), this has erroneously given rise to the notion that aerobic flora would be of importance in the microbial ecology of the bowel.

PROTECTIVE FUNCTION OF THE NORMAL FLORA

Antimicrobial agents may influence the microbial flora of the digestive tract when they are incompletely absorbed following oral administration or when they are excreted in saliva, bile, or mucus (26). Antibiotics are inactivated to a variable extent in the intestines by decomposition (15, 29, 52) or binding (25, 55, 56). The remaining activities of many antimicrobial agents are sufficiently high to disrupt the ecological balance of the microbial flora in the bowel. This may result in colonization by exogenous potentially pathogenic microorganisms (PPMOs) and in the outgrowth of indigenous PPMOs. PPMOs are aerobic or anaerobic microorganisms which may be part of the normal flora of some or many people without causing clinical signs of infection. They may cause infection, however, if the resistance against infection is decreased (as in patients with neutropenia) or if they reach high numbers (such as after the administration of some antimicrobial agents).

In mice which have been treated with streptomycin (6, 34), the infective dose of exogenous microorganisms is between 1,000- and 100,000-fold lower than that in untreated controls. Moreover, in mice which have been treated with streptomycin (6, 34), penicillin (17), or ampicillin (45) and in germ-free mice (18, 24), the concentration of aerobic gram-negative challenge strains in feces is between 1,000- and 100,000-fold higher than the normal level of GNB in the feces of mice (about 10^5 CFU/g of feces).

In short-term experiments, ampicillin disturbed the ecological balance, while on administration for more than 6 weeks, this disturbance could not be obtained. This observation was attributed to the appearance of β-lactamase-secreting bacteria in the bowel (52). In humans as well, administration of antimicrobial agents may result in the outgrowth of resistant PPMOs (30, 33, 39, 65–67). In the pretreatment period of the seven studies in which we used challenge strains, *Klebsiella pneumoniae* and *Enterobacter cloacae* disappeared from the bowel within 5 days (median duration, 2 days) in 41 of 42 volunteers (57–61, 63, 64). All 18 volunteers treated with cefotaxime, clindamycin, or co-trimoxazole became colonized with these strains. Subsequently, these strains remained detectable in feces for variable periods of time up to 3 weeks after treatment (57, 61, 64). Concurrently, cefotaxime and clindamycin increased the concentration of enterococci in feces and all three antimicrobial agents increased the concentration of yeasts in feces.

Pseudomembranous colitis caused by *Clostridium difficile* is almost always associated with the use of antimicrobial agents. Nearly all antibiotics may be implicated (4, 7). Conversely, in

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germfree mice (24) and mice that were treated with antibiotics (20, 53), the abnormally high degree of susceptibility to colonization by aerobic microorganisms was strongly reduced by the administration of murine (20, 24, 51) or even human (24) anaerobic flora. In humans, a dramatic cure was reported in patients suffering from pseudomembranous colitis when fresh fecal suspensions were administered by an intrajejunal tube (9) or by enemas (9, 41, 46).

These data indicate that the normal flora provides protection against colonization by exogenous microorganisms and limits the concentration of indigenous PPMOs.

COLONIZATION RESISTANCE

The term colonization resistance was introduced by van der Waaij et al. in 1971 (50) to indicate the resistance against colonization by exogenous PPMOs (50, 52). The same definition was used by Nord (37) and Barza et al. (5). It is very likely, however, that the flora that provides CR against exogenous microorganisms is identical to the flora that limits the concentration of indigenous PPMOs. Apparently, this was obvious to van der Waaij and Berghuis-de Vries (51) in 1974, when they reported that the concentration of enterococci and yeasts in feces remained unchanged following elimination of *E. coli* by nalidixic acid and concluded that "this indicates that the fraction of the anaerobic microflora which is responsible for the CR of the digestive tract is not affected by the treatment" (51).

Results of our studies support the hypothesis that CR against exogenous and indigenous aerobic PPMOs is provided by the same flora. Following the administration of amoxicillin, cefotaxime, clindamycin, or co-trimoxazole, an impairment of the flora that provides CR was indicated by facilitation of colonization by challenge strains (*K. pneumonia* or *E. cloacae*). In the same volunteers, we consistently observed an increase in the concentration of resistant indigenous GNB, enterococci, and yeasts in feces (57, 61, 63, 64). For this reason we define microbial CR as the "limiting action" of the normal flora on colonization of the bowel by exogenous as well as indigenous PPMOs. This limiting action prevents colonization by exogenous PPMOs and prevents the outgrowth of indigenous PPMOs such as *E. coli*, enterococci, and yeasts.

It should be noted that CR is not provided exclusively by microbial flora. CR is also mediated by anatomical and physiological factors including intact mucosa, salivation, swallowing, secretion of immunoglobulin A, production of gastric acid, desquamation of cells of the mucous membranes, and normal gastrointestinal motility (49). These factors antagonize adhesion of microorganisms to mucous membranes and promote rapid gastrointestinal transit. It appears, however, that anatomical and physiological CR systems are not capable of keeping the concentration of PPMOs under control if the normal flora is absent or disturbed.

FLORA THAT PROVIDES COLONIZATION RESISTANCE

On experiments with germfree mice and mice which were treated with antimicrobial agents, Dubos (16) concluded that the protective flora consists exclusively of obligate anaerobic microorganisms. Enterococci and members of the family *Enterobacteriaceae* would not belong to this flora. His main argument was that strains of mice that were not colonized with these aerobic organisms were completely healthy and had normal resistance to colonization by exogenous microorganisms. Van der Waaij and colleagues (50) reached the same conclusion.

Other investigators have shown that aerobic flora may exert bacterial interference as well. Following oral administration of *Shigella flexneri* to germfree mice, the concentration of *S. flexneri* in feces reached 10^9 CFU/g of feces (18). However, complete suppression of *Shigella* strains by *E. coli* was observed when the two organisms were introduced as the only flora in antibiotic-treated animals (20).

Colonization by *S. flexneri* was limited to 10^5 CFU/g of feces in germfree mice which had been colonized with a cultured mixture of anaerobic bacteria (18). The number of *S. flexneri* in the cecum was reduced from 10^5 CFU/g to below the detection level ($<10^3$ CFU/g) when *E. coli* was administered concomitantly with the cultured mixture of anaerobes (20). From the results of that study it was inferred that *E. coli* acts synergistically with anaerobic flora in limiting the colonization of the bowel by other GNB. In those studies, however, the concentration of *E. coli* in feces was much higher than that in the feces of healthy animals, indicating that the flora that provides CR was not complete. Therefore, results of those studies do not allow investigators to make definite conclusions concerning the protective role of *E. coli* in animals with a normal flora. It appears, however, that *E. coli* may contribute to CR against exogenous GNB in animals with impaired CR.

It has been shown that cefoperazone eliminates anaerobes from the feces of healthy volunteers (5). Nevertheless, the volunteers did not become colonized with challenge strains of GNB. From this it was concluded that anaerobes are not important for CR in humans (5). However, the concentration of cefoperazone in feces was more than 100-fold greater than the MICs for the challenge strains. Moreover, the concentration of yeasts increased 10^6-fold. In our view, this demonstrates that cefoperazone impairs the flora that provides CR.

From our studies it appears that aerobic PPMOs do not contribute to CR in healthy volunteers. Since cefoxitin (21) and clindamycin (57) increase the concentration of members of the family *Enterobacteriaceae*, enterococci, and yeasts in the feces of human volunteers, these three groups of aerobic flora do not limit the concentrations of each other.

Elimination of GNB from the bowel in human volunteers by administration of 20 mg of pefloxacin daily did not induce an increase in the concentration of enterococci or yeasts in feces and did not facilitate colonization of the bowel by a resistant challenge strain of *K. pneumoniae* (57). One of the volunteers in that study was not colonized with GNB or yeasts in the pretreatment period. Nevertheless, he had a normal concentration of enterococci and good CR against a challenge strain of *K. pneumoniae* (57). Results of that study indicated that indigenous *E. coli* is not an essential part of the flora that provides CR against aerobic PPMOs.

Borriello and colleagues (7, 8) developed a model to investigate CR against *C. difficile* in vitro. Growth and toxin production of this organism are inhibited when it is seeded into emulsions of feces from healthy volunteers. This inhibitory action is eliminated by aeration of the emulsion. Apparently, the protective flora in this model consists of anaerobes (7).

Results of that study also support the hypothesis of Dubos (16) that the anaerobic flora that provides CR protects against all PPMOs, aerobic as well as anaerobic.

These data are compatible with the hypothesis that the microbial CR in humans is mainly provided by anaerobic flora. If the flora that provides CR is undisturbed, the potentially pathogenic aerobic flora does not provide a measurable contribution to CR. If the anaerobic flora that provides CR is impaired, the concentration of *E. coli* in feces becomes higher than normal if resistant strains are present. In this situation, *E. coli* may contribute to the CR against exogenous GNB (20, 63).

STUDY DESIGN

Study subjects. The first observations on the influence of antimicrobial agents on CR originated from experiments in animals, mostly mice. Those studies demonstrated the existence of a protective microbial flora. It is unlikely, however, that the composition of the flora that provides CR in mice and their susceptibilities to antimicrobial agents are identical in mice and humans. Moreover, the dosage of antimicrobial agents used in animal models cannot be reliably extrapolated to those required in humans. Accurate extrapolation is of major importance since the influence of antimicrobial agents on CR is dose dependent (31). Therefore, classification of antimicrobial agents according to their influence on CR must be based on studies performed in humans.

Patients are not ideal subjects for these kinds of investigations. Their CR may already have been impaired by their disease (32), stress (14) or previous use of antimicrobial agents. Moreover, it is quite difficult to obtain feces from hospitalized patients regularly. Therefore, volunteers are preferred.

Indicators of CR. Impairment of the flora that provides CR may be indicated by an increase in the concentration of GNB, enterococci, or yeasts in feces or by facilitation of colonization of the bowel by a challenge strain.

Even if antimicrobial agents impair CR, this will not cause an increase in the concentration of PPMOs in feces unless a resistant flora is present, however. For this reason, quantitation of GNB and enterococci may fail to disclose impairment of CR if the concentration of the antibacterial agent in feces is high enough. In such cases, the concentration of yeasts is a useful endogenous indicator of impairment of CR (21, 59). In the case of pefloxacin, impairment of CR has also been disclosed by an increase in the concentration of staphylococci in feces (22).

If the active antimicrobial concentration in feces is greater than the MIC for the prevailing GNB, impairment of CR against GNB can be indicated by outgrowth of a resistant challenge strain. The challenge strain is acquired preferably from surveillance cultures of feces from patients or volunteers who carried the strain during antibiotic treatment but who did not obtain an infection. This ensures that the strain is relatively innocuous and has colonizing ability. The challenge strain should be susceptible to nontoxic antibiotics. This enables immediate treatment in the event that problems arise (64).

It should be emphasized that the challenge strain should be resistant to the active concentration of the antimicrobial agent in the bowel, which may be much greater than the active concentration in blood (22, 43, 58, 59).

Statistical analysis. In most studies, the influence of antimicrobial agents on CR is deduced from their impact on the median concentration of microorganisms in the feces of a group of individuals. However, the antimicrobial susceptibilities of the flora that provides CR may differ among volunteers (29, 52, 63). Therefore, it is preferable to analyze the influence of antimicrobial agents on CR in each volunteer separately. The use of each volunteer as his or her own control has the additional advantage that it improves the possibility of studying the correlation of the influences of antimicrobial agents on several groups of PPMOs (57–61, 63, 64). The number of fecal samples required to obtain statistically significant data depends on the fluctuation of the normal level of microorganisms and the sensitivity which is desired. In general, a 10-fold change in the median concentration of GNB in feces is statistically significant (at $P < 0.05$) if 10 pretreatment samples and 10 treatment samples are used (63).

INFLUENCE OF ANTIMICROBIAL AGENTS ON THE POTENTIALLY PATHOGENIC FLORA OF THE BOWEL

The influence of antimicrobial agents on the concentrations of PPMOs in the bowel is determined both by their influence on the flora that provides CR and by their direct influence on PPMOs. This creates the four possibilities described below.

(i) Neither potentially pathogenic flora nor the flora that provides CR is inhibited. In this case the concentration of microorganisms in feces does not change. Antibiotics that are used for the treatment of urinary tract infections have the best chance of meeting these requirements. If absorption is complete and excretion in urine is rapid, such agents may be effective in the urine, although levels in tissues and feces are very low. An example of this possibility has not yet been published. In contrast to our expectations, cephradine given at 500 mg twice daily induced a slight impairment of CR in four of six volunteers (60). In the other two volunteers, CR was not impaired.

(ii) The potentially pathogenic flora is inhibited, whereas the flora that provides CR is not. Susceptible PPMOs are eliminated, while resistant flora will not grow out, since the CR is not disturbed. This is called "selective decontamination" (12, 51). Selective decontamination of the bowel from GNB can be achieved by administration of 20 mg of pefloxacin daily (57).

(iii) The flora that provides CR is inhibited, whereas the potentially pathogenic flora is not. In this case, impairment of CR enables outgrowth of resistant PPMOs. Amoxicillin, cefotaxime, and clindamycin exemplify this. The differences in their spectra of activity against aerobic flora are reflected in their impacts on the aerobic flora of the bowel. Clindamycin causes an increase in the concentration of GNB, enterococci, and yeasts in feces (57). Amoxicillin causes an increase in the concentration of GNB and yeasts in feces, but the concentration of enterococci is generally reduced. Enterococci also grow in people who harbor or acquire penicillin-resistant strains (63). Cefotaxime causes an increase in the concentration of enterococci and yeasts in feces. The concentration of GNB in feces decreases in general, since cefotaxime-resistant GNB are not prevalent. However, colonization by resistant challenge strains such as *E. cloacae* is facilitated (64).

(iv) Both potentially pathogenic flora and the flora that provides CR are inhibited. This might be called "unselective decontamination." Susceptible PPMOs are inhibited, but the concentration of resistant microorganisms increases. The existing impairment of CR goes unnoticed in patients who do not harbor or acquire resistant PPMOs. It should be noted that the active antimicrobial concentration in feces may be much higher than the concentration in plasma (43, 58, 59). For this reason, it is possible that some strains cannot disclose an impairment of CR, although they are resistant by conventional criteria. For example, erythromycin eliminates GNB from the bowel (62), although GNB are classified as erythromycin resistant.

DISCUSSION

Recent data support the hypothesis that aerobic PPMOs do not contribute to the normal microbial CR. In principle, it should therefore be possible to treat infections without causing superinfections by selecting those agents which are active against PPMOs but that spare the flora that provides CR. It was assumed that such agents were available (12, 47, 48). Unfortunately, it now appears that few, if any, of the available antimicrobial agents spare the flora that provides CR in humans. It should be noted, however, that impairment of CR will not induce outgrowth of PPMOs if the active concentra-

tion of the antimicrobial agent in the bowel is effective against the prevailing PPMOs. Thus, it can be predicted that the risk of superinfections will not be the same with all antimicrobial agents. The risk of gram-negative superinfections is probably higher following amoxicillin and clindamycin treatment (which cause outgrowth of GNB in most volunteers) than following treatment with quinolones (which rarely cause an outgrowth of GNB). However, this hypothesis has not yet been confirmed in prospective studies.

The importance of sparing of the CR ("selectivity") by antimicrobial agents used for prophylaxis has been overestimated. From experiments in animals it was predicted that selectivity is compulsory in the prevention of superinfections (48). This notion appeared to be corroborated by the observation that prophylaxis of infections in neutropenic patients is more effective with new regimens carrying the flag of selective decontamination than with older regimens which are known to impair CR (12). However, the initial regimens of selective decontamination in neutropenic patients contain co-trimoxazole (12), and most regimens used in patients on mechanical ventilation contain cefotaxime (54). It has now been proven that neither of these agents spares CR (61, 64). Consequently, the very success of these regimens implies that selectivity is not necessary for efficacy in decontamination of the bowel.

In retrospect, the simple explanation for the success of these new regimens is that their antimicrobial activities in the bowel were high enough to prevent the outgrowth of the prevailing PPMOs. Because they are not selective, these regimens may cause the outgrowth of resistant strains, as has been observed in practice (23, 27, 28).

Even if the prophylactic regimen were selective, the outgrowth of antibiotic-resistant strains would occur if the CR of the patient was previously impaired by other factors, such as illness (32), stress (14), or previous use of antimicrobial agents. An effective and reliable regimen must therefore provide coverage against all prevailing PPMOs. Ideally, this should be achieved with selective agents, whereas selectivity is not mandatory for efficacy.

Failures of decontamination should be analyzed meticulously. If they occur frequently, the regimen should be adapted.

The bowel is an important reservoir of PPMOs (3, 11). Many "nosocomial" gram-negative infections are caused, in fact, by strains originating from a patient's bowel. Although some of these strains may be acquired by cross-contamination, many of these are present before admission of the patient to a hospital ward (19, 38). This explains why hygienic measures such as hand disinfection cannot completely prevent these infections (19, 38). The logical way to address this problem is by concomitantly eliminating these strains from the bowel with suitable antimicrobial agents. This perception has been applied successfully in terminating hospital epidemics with multiresistant GNB (1, 10, 44).

It appears that the bowel is an independent pharmacokinetic compartment. The active concentrations of antimicrobial agents in feces may deviate strongly from their active concentrations in plasma (43). It should be noted that there is no relation between the active concentrations of antimicrobial agents in the bowel and the fraction of the dose that is absorbed. For example, pefloxacin reaches high concentrations in feces, although it is almost completely absorbed (22, 58, 59). Conversely, extensive inactivation may cause in feces unexpectedly low levels of activity of drugs that are poorly absorbed (25, 55, 56). It is therefore of major interest that more data on the active concentration of antimicrobial agents in the bowel be obtained. This will improve the possibility of making a rational selection of antimicrobial agents if traditional regimens fail to eliminate a multiresistant strain from the bowel.

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